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## EFFECT ON CHESTNUTS OF SUBSTANCES INJECTED INTO THEIR TRUNKS

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These observations are based on tree injections made during the summers of 1912, 1913, 1914, and 1915.<sup>1</sup> All the trees were orchard chestnuts: Paragon scions grafted on *Castanea dentata*.

### TREES INJECTED WITH DYES

Some evidence concerning the path of the injected solution in the tree was obtained from stains. In 1912, 0.01 percent solutions of eosin, methyl green, and Congo red were injected into the trees. Although the holes were cut into the heartwood, the stains were found in the vessels of the youngest, or last annual ring; from here the stain spread slightly through the surrounding tissue. It was found that the stain descended as well as ascended in these vessels. In the roots the path of the stain was not followed to the root tips; it was found in the ring of vessels. In the small branches and twigs of the tree the stain often encircled the wood, whereas in the trunk lower down it had been seen in patches only in the last year's ring of vessels. The dyes varied in their effect on the trees. The eosin stain passed into the leaves of the injected trees and was toxic in the dilution used. Methyl green was not a good stain in that the color showed a tendency to fade. Illustrations of sections of these injected trees are in the report of the physiologist of the Pennsylvania Chestnut Blight Commission for the year 1912.<sup>2</sup>

In 1913, the non-toxic dyes, methylene blue, Congo red, and trypan blue, in 1/40 percent solution were injected for twenty days into six small trees, the injections starting April 16. At first the solutions flowed rapidly into the trees, especially the methylene blue, then more slowly, and had practically ceased going in when the injections were stopped. The trees were cut down in October. Two of these trees were infected with the

<sup>1</sup> Rumbold, C. The injection of chemicals into chestnut trees. Amer. Journ. Bot. 7: 1-20. 1920.

<sup>2</sup> Rumbold, C. Report of the physiologist. Report of the Pennsylvania Chestnut Tree Blight Commission, Harrisburg, July 1 to December 31, 1912, pp. 45-47, figs. 43-47. 1913.

(The Journal for January (7: 1-43) was issued February 25, 1920.)

chestnut blight fungus; one had just been girdled at the base of the tree. The stain extended into the branches and roots, sometimes but part way, sometimes to the beginning of the year's growth. In no case did it extend beyond the beginning of the new year's growth of twigs and roots. At the point of injection it had penetrated three annual rings of wood and was found in the bark. However it was soon confined to the last annual ring in the neighborhood of the vessels. The girdled tree showed the stain passing through the diseased area by way of the vessels and tracheids; traces of it were found in the bark where disintegration had just begun. It will be noticed that the results of injection in 1912 and 1913 differed. No experiments were undertaken to discover the cause of this difference.

As stated before, most of the dye passed through the xylem elements of the last formed annual ring. At first it entered the large spring vessels, which appeared, however, not always to be the carriers. The summer tracheae or vessels, which in cross sections of Paragon chestnut form characteristic flame-like lines of pores diverging from the spring vessels, were deeply stained, and so were the tracheids. The trees injected with methylene blue showed ragged tissues and holes in the neighborhood of the spring vessels. There were traces of dye found in some of the vessels of the new growth of wood near the point of injection, but generally this new growth was unstained.

A microscopic examination of the xylem cells showed that the dye was retained by the walls of the vessels and tracheids through which it passed.

These phenomena (the decreasing size of the injected area and the gradual dilution of the solution as the distance from the point of injection increased) were observed when solutions of salts were injected into growing trees. Though the paths could not be followed as easily as when dyes were used, they could be traced, often, by a formation of abnormal bark tissue which disappeared as the distance from the point of injection increased. When a "killing" solution was injected the path was marked on the trunk by vertical strips of dead tissues. Those twigs and branches whose vascular system entered this path were killed; often but one side of a branch was affected. All stages of reaction to an injection could be seen in a tree: dead tissue at the point of injection in the trunk, dead or falling leaves on the branches nearest the injection hole, spotted leaves on branches higher up the tree, and no signs of injection visible in the top of the tree.

#### TIME OF INJECTION AND DILUTION OF SOLUTION

The way in which a tree was affected depended both on the time of the injection and on the dilution of the chemical solution. Concentrated solutions acted more quickly than dilute ones and generally were injurious.<sup>2</sup>

<sup>2</sup> "Experiments on the  $P_H$  or true acidity values of normal and cankered chestnut bark adjacent to the cambial layer show that healthy chestnut bark has a  $P_H$  of about 4.8,

### Alkali Metals

In September the effects of an injection of lithium carbonate  $1/20$  G.M. were visible in the leaves three days after the injection started. The leaves nearest the point of injection were first affected, those furthest distant last. When the dilution was  $1/200$  G.M. or weaker, the leaves first affected were at the ends of the branches; in August they were those nearest the burs. Trees injected in late summer with a  $1/20$  G.M. dilution produced normal leaves in the following spring at the normal time, but after the leaves were full grown they gradually showed the characteristic lithium curling and spotting (Pl. I, fig. A), although no more injections had been made in the trees. The other lithium salts acted in the same manner.

What happened in the case of the lithium salts, happened to some extent with the other alkali metals (ammonium compounds excepted). Sodium solutions  $1/20$  G.M. in strength produced effects like those of the lithium salts; but when diluted to  $1/200$  G.M. they did not blotch the leaves or affect the young bark, so that the presence of unusual quantities of sodium in the branches could not be vouched for. Potassium salts behaved like the sodium. It was observed in October that the sodium-, potassium-, and ammonium-injected trees ( $1/100$  G.M. and  $1/200$  G.M.) generally lost the leaves on the ends of the branches near the burs first, and that this leaf fall was previous to the fall on the water-injected trees. The ammonium salts: chloride  $1/200$  G.M., carbonate  $1/100$  G.M., and hydroxide  $1/100$  G.M., also appeared to affect the leaves on the ends of the branches, causing them to drop; the sulphate  $1/200$  and  $1/500$  G.M. blotched the leaves. The normal growth of the trees was not seriously affected by the injection of the alkali metals.

### Heavy Metals

Of the heavy metals injected, potassium chromate and bichromate and copper sulphate and chloride showed their effects most quickly. The chromates were more toxic and spread through the tree more quickly than the copper salts; the bichromate was more poisonous than the chromate.

The leaves of the tree injected with the chromate solutions came affected in 48 hours. The veins of the leaves browned first, then the leaves curled upward, dried, and dropped off. New leaves formed, but they in turn fell. The dilution  $1/10000$  G.M. of potassium chromate behaved like the  $1/20$  G.M. copper salts. With the exception of those trees injected with this latter dilution, the trees of this series were almost bare in August, whereas samples of cankered bark have shown  $P_H$  values as low as 3.24. A  $P_H$  of 3.24 represents an acidity about 23.7 times that of 4.8. When  $N/100$  and  $N/1000$  alkalies are injected into the tree the acidity automatically increases very slightly as a kind of immunity to offset the effect of the alkali. When larger quantities of  $N/10$  alkali are injected the sap becomes decidedly alkaline and the tree dies. The details of these investigations will appear later in the *Journal of Agricultural Research*. (C. Rumbold, M. R. Meacham and S. F. Acree.)

Ten days after being treated with potassium bichromate  $1/1000$  G.M., the back of the tree cracked along the edges of the path of the solution. In July and August, *Penicillium* sp. grew luxuriantly in these cracks and in the points of injection. The following year all the trees injected with the chromate solutions were dead.

The day after an injection of copper sulphate  $1/20$  G.M. was started, the leaves began to turn brown, those nearest the point of injection first. Copper chloride  $1/20$  G.M., zinc chloride  $1/20$  G.M., and barium chloride  $1/20$  G.M. acted almost as quickly. All of these were "killing" solutions. As previously stated, the paths these solutions took up and down the tree could be followed by the visible killing of the tissues. The region they passed through was a narrow one, but little wider than the hole made for the injection. Those twigs and branches whose fibers entered this path showed dead leaves. The leaves were the first to show the effects, those nearest the point of injection browning first. A smell of decaying plant tissue became noticeable (in the case of  $\text{Cu}_2\text{SO}_4$   $1/20$  G.M. in 10 hours, during which time 1 liter had been injected), which sometimes continued for one and two days. The dying leaves did not become crisp until some time after they had browned, in one case not until four days after browning. Environmental conditions probably influenced this phenomenon. Eventually all the leaves on the trees died, and soon those on the parts of the trees not included in the paths of the solutions fell off. The leaves dropped as they would in the autumn. The denuded branches quickly produced new leaves, so that trees injected in August had full-sized green leaves in December. The dead leaves still hung on the injected branches, rendering them conspicuous. The following spring these trees leafed, and produced fruit like the surrounding trees. The branches which had been injected were dead. The effect on the other parts was as though the trees had been severely pruned.

#### Colloidal Metals

The solutions of the heavy metals proved detrimental to the normal growth of the trees. The colloidal metallic solutions were exceptions. Examination of the injected trees indicated that most of the injected colloids stayed in the trunks near the place of injection.

#### Carbon Compounds

Two of the carbon compound solutions proved very toxic when injected. Four-tenths percent formaldehyde<sup>4</sup> affected the trees much as did the stronger concentrations of the copper solutions, but more severely for the reason that formaldehyde made broader paths when passing up the trunks. The trees above the point of injection were dead the following spring, but produced suckers from the base of the tree and from buds near the base.

<sup>4</sup>Schering.

Meta-cresol 1/1000 G.M. killed the tissues as it passed up and down the tree. The midribs and veins of the leaves browned and exuded a smell of creosote. Finally they turned black and shriveled, hanging to the twigs as though scorched by fire. Along the sides of the path of the solution callus formed. The bark peeled from the injected area and exposed the wood. Outside this path the tree was unaffected (Pl. I, figs. C and D).

The dilutions of the carbon compounds injected, with the two above noted exceptions, did not, apparently, seriously affect the normal growth of the trees, though some of them caused blotching of the leaves.

#### Extracts

Canker extract killed the trees. Water extract of healthy bark did not affect them.

#### Water

Water injected into trees for three succeeding years apparently in no way modified their growth.

#### DISCOLORATION OF LEAVES DUE TO INJECTION

Some of the solutions injected affected the leaves in so marked a manner that one could tell from the type of blotching what base had been introduced.

Lithium produced the most characteristic blotches of all the substances. These blotches appeared irrespective of whether a carbonate, hydroxide, chloride, nitrate, or sulphate was introduced. Usually the tip and the edge of the leaf between the veins turned a reddish brown color, giving the leaf a scalloped appearance (Pl. III, fig. A). Sometimes, however, these spots appeared in the parenchyma in the middle of the leaf. A dark line separated the green from the brown area. The leaf curled upward. As more lithium accumulated, the discolored area advanced toward the midrib. The base of the leaf was the last to turn brown.

Sodium carbonate 1/20 G.M. killed the leaf parenchyma in somewhat large irregular areas, which sometimes were in the central part of the leaf extending across veins and leaving the leaf edges green. The division between green and brown areas was sharply defined. Dilute solutions of sodium salts did not blotch the leaves. The potassium salts in the dilutions used in the injections did not blotch the leaves.

Ammonium compounds did not brown the leaves, but ammonium sulphate 1/200 G. M. and 1/500 G.M. caused a wrinkling or frilling of the leaf edges. This frilled area became translucent and later brittle, and the network of small veins showed prominently. Occasionally these wrinkled areas looked bleached, and were surrounded by a dark green band.

The colloidal metals did not visibly affect the leaves.

Concentrated heavy metal solutions produced three varieties of discolored leaves; one, a browning of the midrib and veins, which gave the

leaves a finely checked appearance. The parenchyma browned last. The leaves then became dry and curled upward. There was another kind of discoloration characteristic of these solutions which appeared on leaves distant from the point of injection, or at a point where the solutions injected were diluted. Irregular brown spots appeared on the edges of the leaves which spread gradually toward the green petiole. The line of demarcation between brown and green areas was sharply defined. Such leaves were found on all trees injected with heavy metals. This effect in turn was quite different from that produced on leaves in the uninjected parts, of trees treated with concentrated solutions, where a gradual bleaching appeared.

The manner in which formaldehyde 4/10 percent and meta-cresol 1/1000 G.M. affected leaves has been described. While meta-cresol proved so toxic, para-cresol 1/1000 G.M. produced no apparent effect on the leaves.

Those carbon-compound-injected trees which had discolored leaves showed two variations of discoloration. Para-nitro-phenol 1/500 G.M. browned the midribs and veins of leaves near the point of injection. Those leaves gathered from more distant parts showed light brown blotches on the edges which gradually advanced toward the base of the leaf. (The leaves, as far as appearance was concerned, could have been taken from a tree injected with  $\text{HgCl}_2$  1/1000 G.M.) Trees injected with the 1/1000 G.M. solution of para-nitro-phenol also showed these two varieties of discolored leaves. Ortho-nitro-phenol 1/1000 G.M. produced effects on leaves resembling those on ammonium-sulphate-injected trees, the leaves having translucent, brittle, frilled edges.

Picric acid 1/1000 G.M. caused the appearance of blotched and frilled leaves; citric acid 1/50 G.M., of blotched leaves; citric acid 1/500 G.M., of blotched and frilled leaves; acetic acid 1/500 G.M., of blotched leaves; formic acid 1/1000 G.M., of blotched leaves; salicylic acid 1/5000 G.M., of blotched and frilled leaves; pyrogalllic acid 1/1000 G.M., of blotched leaves, the entire leaf finally turning a bright yellow and dropping off, as well as of frilled leaves; phloroglucine 1/1000 G.M., of frilled leaves; pyrocatechin 1/1000 G.M., of frilled leaves.

A possible explanation for these three variations in the discoloration of the leaves on an injected tree is that the leaves became impregnated in the course of the solution's spread with varying dilutions of the injected substance, those at a distance being impregnated with a much more dilute solution than those near the place of injection. The more concentrated solutions killed the tissues as they passed, thus browning the midribs and veins of the leaves, leaving the parenchyma green. When sufficiently dilute they flowed into the leaves without apparent harm, but gradually accumulated through transpiration in the parenchyma cells until a poisonous effect was produced. The third variation, that in which the leaf edges wrinkled or frilled, may be the effect not of the substance originally injected,

but of by-products resulting from injuries caused by its presence in the lower parts of the tree. These three variations did not appear on every injected tree; sometimes there was but one kind, sometimes there were but two kinds of discolored leaves.

#### EFFECT ON TRUNKS

The holes made for the injections usually were filled with grafting wax after the removal of the injection tubes. A callus growing from both sides of the wound gradually closed it, leaving a small slit hardly noticeable on the tree. Sometimes this callus forced the wax from the hole, sometimes completely closed it in. It was found on examining felled trees that callus might cover the injection wound while an air space extended from the point of injection up and down the tree trunk between the outer bark and the wood (Pl. III, fig. B). This hole or tunnel was caused by the failure of the new annual ring to grow at that point, the cambium layer having been killed by the injected fluid. Such holes, first noticed in trees injected with lithium salts, were found to be a somewhat usual result of injection. Trees treated with meta-cresol 1/1000 G.M., formaldehyde 0.4 percent, potassium bichromate 1/1000 G.M., or mercuric chloride 1/1000 G.M., showed these holes in marked degree in that the bark cracked and peeled away from the treated area. The lithium-injected tree, first noticed, had been injected in the late fall and the injection wound had been left uncovered. In the spring of the following year, this hole was found filled with water below the point of injection. It was uncovered by cutting away the bark. There was no chestnut blight infection found and callus had formed along its sides. It extended from a point at the base of the tree about three feet below the point of injection to a point somewhat less than three feet above the hole. It was thought that some of these holes might be formed beneath the bark by the eroding effect of the extraordinary amount of foreign fluid passing through a narrow channel rather than by the toxic character of the fluid. Trees injected with methylene blue showed this disintegration in a less marked form. Primarily, the nature of the solution injected determined the formation of these holes and their size, for an examination of the injected trees showed that weak acids, water, and extracts did not produce such holes. A tree into which para-nitro-phenol 1/1000 G.M. had been injected, and in which one injection ran for more than five weeks, showed short and rather narrow holes. The colloidal metals produced no holes, nor was there an abnormal growth of tissue.

The "killing" solutions produced no stimulation of growth further than the callus which cut off the dead tissue from the living. Solutions more dilute did not kill the tissue outright, but caused the formation of wound tissue in the growing annual ring and bark.



## EFFECT ON FRUITS

All the injected trees with the exception of those treated with the concentrated solutions of the heavy metals and formaldehyde produced a normal appearing crop of nuts.

There was no sign of a stimulation of the trees by the substances injected further than that the nuts growing on trees treated with the alkali metals in general appeared somewhat larger and glossier than those on trees injected with water or carbon compounds. Lithium was found in the nuts gathered from the trees injected with the lithium salts. The contents of the nuts gathered from the other injected trees were not tested further than by a superficial feeding experiment with white rats<sup>5</sup> to test their possible poisonous effect. In view of the fact that lithium was found in the nuts, it seemed possible that some of the other injected substances had found their way into the fruits. The amount of poison in them must have been extremely small since they did not appear to injure the rats' health. Another indication of this lack of toxicity was a count made of the wormy nuts gathered from treated and untreated trees in the orchard. This count showed the percentage of wormy nuts to be the same for both classes of trees.

It seems possible, judging from the varying results of the injections made in the spring and fall, that the amount of injected substance which finally reaches the nuts can be influenced by the time of injection. The late summer injections quickly affected the chestnut fruits, as shown by the spotting of the burs and neighboring leaves when injected with lithium.

For the sake of brevity the substances injected are arranged as carefully as possible in groups according to the effect they produced on the trees during the summers of experimentation. Very often the trees did not respond in the same degree to injections of the same chemical so that it was difficult to judge its general effect, and possibly some of these dilutions of chemicals could be put in two groups.

*No apparent effect on trees*

Water

Water extract of healthy chestnut tree bark

Congo red 1/40 percent

Trypan blue 1/40 percent

Colloidal cuprous hydroxide 1/3300 G.M.

Colloidal metallic silver 1/6400 G.M.

Methyl alcohol 1/100 G.M.

Acetic acid 1/3000 G.M.

Formic acid 1/6000 G.M.

Lactic acid 1/1000, 1/2000 G.M.

Anilin sulphate 1/1000 G.M.

Sodium carbolate 1/1000 G.M.

Phenol Sodique, 1 cc. to 1000 cc. H<sub>2</sub>O

Para-nitro-phenol 1/10000 G.M.

Para-cresol 1/1000 G.M.

Thymol 1/3000 G.M.

Oil of bitter almonds 1/10000 G.M.

<sup>5</sup> Chestnuts were gathered from each injected tree and kept separate in labeled paper bags. Twelve rats were fed regularly with the chestnuts. A day of chestnut feeding (the nuts for the day being those gathered from trees injected with a particular chemical) alternated with one of bread, milk, and grain.

The ammonium compounds 1/500 G.M.	Formic acid 1/1000 G.M. Citric acid 1/50, 1/500 G.M.
<i>Apparently a slight stimulant</i>	<i>Detrimental (death of injected part of tree or of whole tree)</i>
The weaker dilutions of the alkali metals	Copper sulphate 1/20 G.M.
Para-nitro-phenol 1/1000 G.M.	Copper chloride 1/20 G.M.
Picric acid 1/10000 G.M.	Zinc carbonate 1/20 G.M.
<i>Slightly detrimental (blotched leaves, death of cambium near the point of injection)</i>	Mercuric chloride 1/1000 G.M.
Para-nitro-phenol 1/500 G.M.	Potassium chromate 1/1000, 1/10000 G.M.
Ortho-nitro-phenol 1/1000 G.M.	Potassium bichromate 1/1000, 1/10000 G.M.
Picric acid 1/500, 1/1000 G.M.	Barium chloride 1/20 G.M.
Pyrocatechin 1/1000 G.M.	Alkali metals 1/20 G.M. (NaCl 1/50 G.M.)
Pyrogallie acid 1/1000, 1/500 G.M.	Formalin 0.4 percent
Phloroglucine 1/1000 G.M.	Acetic acid 1/100 G.M.
Benzoic acid 1/500 G.M.	Formic acid 1/100 G.M.
Phenol 1/1000, 1/500 G.M.	Lactic acid 1/100 G.M.
Copper sulphate 1/100 G.M.	Anilin sulphate 1/100 G.M.
Lithium salts 1/100 G.M.	Meta-cresol 1/1000 G.M.
Ammonium compounds 1/100 G.M.	Benzoic acid 1/500 G.M.
Sodium chloride 1/100 G.M.	Salicylic acid 1/100 G.M.
Eosin 1/40 percent	Water extract of chestnut blight canker
Methylene blue 1/40 percent	
Acetic acid 1/1000 G.M.	

## SUMMARY

For four years observations have been made on the effect of chemical solutions injected into the trunks of chestnut trees.

1. Usually it was found that the visible effect of a solution on a tree varied with the distance from the point of injection.
2. The effect varied with the dilution of the solution and the month in which the injection was made.
3. In general the effect of the injection of the alkali metals was not detrimental to the trees; injection of heavy metals was detrimental; colloidal metals were not detrimental; organic compounds were not detrimental; water extract of chestnut blight canker was detrimental, healthy bark extract was not.
4. Many of the bases produced characteristic discoloration of the leaves.
5. Lithium was found in the nuts gathered from lithium-injected trees. The nuts gathered from the remaining trees were not tested sufficiently to show positively whether or not they contained any of the injected chemicals.\*

THE EFFECT OF THE INJECTED CHEMICALS ON THE FUNGUS  
*ENDOTHIA PARASITICA*

The results of the injections on the growth of the chestnut blight canker on the chestnut tree have been so uncertain and varied that, were it not for the fact that the work must stop for the present, no results would be mentioned.

It seems best to give a history of the results as they presented themselves.

The first indication of an effect from the injected chemicals on the fungus was in the summer of 1913. The trees which had been injected in 1912 had been inoculated with the chestnut blight fungus in the fall. The fungous growth from these inoculations on those trees injected with alkali metals had an abnormal appearance. However, the fungus continued to grow and eventually killed the trees. This abnormal appearance of the fungus together with the fact that the alkali-injected trees had, as a whole, a thrifty look led to the decision to put more emphasis upon the injection of the alkali metals.

In 1914, measurements were made of the cankers caused by the inoculations of 1913. These showed that the cankers on the control trees averaged the same size as those on the alkali-injected trees. The measurements of the cankers on the other injected trees gave confused results. As a whole the injected trees had larger cankers than the uninjected.

In 1915, a dead canker was noticed on a tree, no. 185 E, which had been injected with lithium hydroxide in April, May, and June, 1913, and in June and July, 1914. The dead canker was not noticed at first for the reason that dead bark covered the area (Pl. IV, fig. A). Not until this bark was removed (as one would remove the scab from a healed wound) was it noticed that a healthy callus had cut out the cankerous growth (fig. B). This same effect was noticed on a tree injected with sodium carbonate and on a thymol-injected tree. In 1916 these trees again became infected, and in 1917 the new chestnut blight cankers on them were growing at the normal rate.

In the meantime a better method of injecting the trees had been devised (Pl. IV, fig. C). Injections were made on forest trees as well as on small orchard trees.

In 1916 the injections were made with lithium and sodium salts only. The injections were made in three different regions. One set of trees was injected in April, May, and June, the second in June, July, and August, the third in August, September, and October. The results of these injections showed in 1917 that sodium salts were not as effective as lithium salts. The lithium injections made in April, May, and June seemed to have the greatest effect, in that the cankers were not growing vigorously and the trees had started to form a callus about the diseased areas. All the check trees were dead at the time of the inspection. Those injections made in August, September, and October appeared to have had the least effect. In no case

had an injection definitely stopped the growth of a canker. No further inspection has been given these trees.

#### SUMMARY OF RESULTS

This and the preceding paper<sup>5</sup> constitute a report on an attempt made to answer by experimentation the following questions:

1. What substances can be injected into living chestnut trees?
2. When can they be injected?
3. Where does the injected material go?
4. What is the effect on the tree?
5. What is the effect on *Endothia parasitica* growing on the tree?

A compilation of the records of injections made in living chestnut trees during the growing seasons for five years showed:

1. That the trees possess a considerable capacity for absorbing solutions of substances. Solutions of organic compounds went into the trees more readily than solutions of inorganic compounds, the "true solutions" more readily than the colloidal. Injected solutions, with a very few exceptions, were absorbed more readily than injected water. In the dilutions used in these experiments, the more concentrated the solutions were, the more readily they were absorbed by the trees.

2. In southeastern Pennsylvania, June was the best month for injection in so far as rate of intake was concerned; then came July, May, August, September, October, and April. The rate of intake varied more in April, May, and June than in the summer and autumn months, but obviously was dependent upon the local weather conditions.

3. Examination of the trees showed that the injected solutions as a rule passed through the vessels of the youngest annual ring of wood up and down the tree trunk in a zone whose width was usually but little more than that of the injection hole. They passed into the branches and leaves, and in the case of the lithium salts into the nuts. They passed into the roots.

4. In general, the injection of the alkali metals was not detrimental to the trees; injection of heavy metals was detrimental; colloidal metals were not detrimental; organic compounds were not detrimental; water extract of chestnut blight canker was detrimental, healthy bark extract was not. The effect varied with the dilution of the solution and with the month in which the injection was made. Many of the bases produced characteristic discolorations of the leaves. Usually the visible effect of a solution upon a tree varied with the distance from the point of injection. The injections can cause the appearance of pathological xylem in the tree trunks.

5. This work is not completed and the results are inconclusive. Dilute

<sup>5</sup> Rumbold, C. The injection of chemicals into chestnut trees. Amer. Journ. Bot. 7: 1-20. 1920.

solutions of lithium salts injected in the spring months may have an effect on the chestnut blight fungus in that the growth of the cankers on the injected trees appeared to be checked somewhat and the trees showed a tendency to form a callus about the canker.

INVESTIGATIONS IN FOREST PATHOLOGY,  
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### EXPLANATION OF PLATES III AND IV

#### PLATE III

FIG. A. Tree no. 185E. An 8-year-old, grafted tree 4.85 m. high, 8 cm. in diameter. Injected April 15 to June 25, 1913, with 10 liters of lithium hydroxide 1/500 G.M. Leaf collected June 20, 1913. This tree produced many large nuts in the autumn. The shaded areas in the illustration indicate the brown portions of the leaf.

FIG. B. Tree no. 21C. A 16-year-old grafted tree 5.5 m. high, 1.1 cm. in diameter. Injected June 20 to October 16, 1913, with 26 liters of lithium carbonate 1/500 G.M. A diagrammatic drawing showing a cross section of portion of the trunk: a. Holes running up and down the trunk caused by the death of the cambium layer in the path of the injected alkali. b. The irregular year ring of wood formed during the injection period.

FIG. C. Tree no. 114E. A 9-year-old grafted tree, 46 m. high, 9 cm. in diameter. Injected May 9 to 15, 1913, with 14½ liters of meta-cresol 1/1000 G.M. The branch was cut August 15. Callus had formed along the edges of the paths of the solution. a. Diagrammatic drawing of cross-section of small branch. b. This year's ring of wood, normal in structure. c. The edge of the creosote stain. All tissue reached by the creosote was killed.

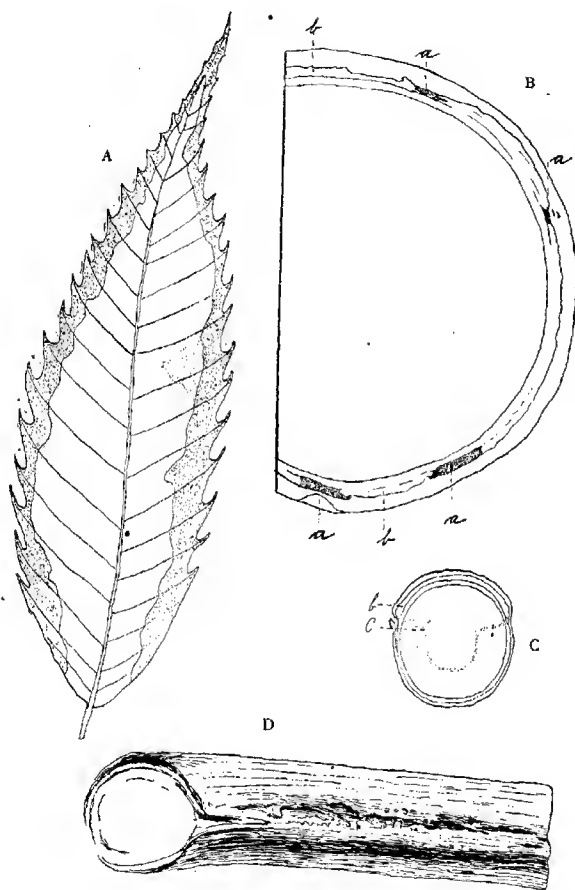
FIG. D. Tree no. 114E. Small branch showing an edge of a path of injected creosote solution. Normal callus separated the living bark tissue from the injected dead tissue.

#### PLATE IV

FIG. A. Tree no. 185E. An 8-year-old grafted tree, 4.85 m. high, 8 cm. in diameter. Injected April 15 to June 25, 1913, with 10 liters of LiOH 1/500 G.M. Injected again June 11 to June 17, 1914, with LiOH 1/200 G.M., and from June 26 to July 27 with 2 liters of LiOH 1/100 G.M. solution. Tree inoculated with *Endothia parasitica* October, 1913. Canker photographed October, 1915, when it was noticed that the canker had stopped growing.

FIG. B. Tree no. 185E. Same canker as above, photographed in November, 1915, when the dead bark formerly covering the canker had been pulled off. The clean, healthy callus which had "cut out" the fungus was thus disclosed. On the side branch can be seen the check made at the time the tree was inoculated. At the base of the photograph can be seen the upper part of a canker caused by a natural infection at the fork of a branch. This canker also had been "cut out" by a callus.

FIG. C. A method of injecting trees of any diameter. Link chains tightened by turnbuckles hold the rubber corks to the trees. Glass T-tubes thrust through the corks introduce the liquid into the injection holes. A tempered steel tube shaped like a cork-borer makes the hole for the injected solution. It can be driven into the tree through the horizontal arm of the T-tube after the apparatus is in place. A piece of rubber tubing is put on the free end of the horizontal arm of the tube, and the solution is cut off with a pinchcock after the drill is removed.



• RUMBOLD: EFFECT OF CHEMICALS ON ENDOTHEIA





A



B



C

RUMBOLD: EFFECT OF CHEMICALS ON ENDOTHRIA





## SUBALPINE LAKE-SHORE VEGETATION IN NORTH-CENTRAL COLORADO

FRANCIS RAMALEY

### INTRODUCTION

Very little has been published on the shore vegetation of lakes in Colorado. Brief references were made to lakes of the Pike's Peak region a number of years ago by Clements (1, 2). A somewhat extended account by the writer and W. W. Robbins (9) described the associations at Redrock Lake, Boulder County, Colorado, in the subalpine zone. Later, a short paper (5) pointed out certain features of shore vegetation in the montane zone. Recently Dr. Robbins has given a most careful and illuminating description (11) of a number of lakes in the montane zone near Tolland, Colorado. The present writer, dealing with sedges of northern Colorado, has named and characterized (7) certain of the plant associations of lake shores at different altitudes. In a paper by Dodds (3) on the plankton crustacea of Colorado lakes there are some references to vegetation and a very good account of physiography and climate.

The following pages give the results of a study of subalpine lakes, the study carried on chiefly from the University of Colorado Mountain Laboratory (8) at Tolland, Colorado, during the last ten years. A later paper will take up alpine lakes.

### PHYSIOGRAPHY

The area in which lake-shore vegetation has been studied is a strip about 5 miles wide, along the eastern slope of the continental divide and extending from the southern boundary of the Rocky Mountain National Park south to Parry Peak, a distance of 24 miles. About 50 of the subalpine and alpine lakes of this district have been visited, and also a few on the western slope, in Grand County (see maps, figs. 1 and 2). The subalpine lakes more carefully studied are listed below together with altitudes in feet above sea level. These lakes are all of small size, the largest scarcely more than a half mile in length.

Lakes in Boulder County: Redrock Lake (10,100), Brainard Lake (10,350), Long Lake (10,500), Silver Lake (10,200), Emerald Lake (11,250), Dixie, or Jenny, Lake (11,000).

Lakes in Grand County: Corona Lake (11,165), Corona Reservoir (11,350), Lake Epworth (11,250).

Lakes in Gilpin County: Forest Lakes (10,800-10,900), Arapahoe Lakes (10,700-11,200), Crater Lakes (10,400-11,000), Echo Lake (11,072), James Peak Lake (11,090).

Lakes in Clear Creek County: Steuart Lake (11,350), Reynold's Lake (11,350), Loch Lomond (11,140).

The continental divide in the area studied is, for the most part, about 12,000 feet above sea level, with a few passes slightly lower and certain

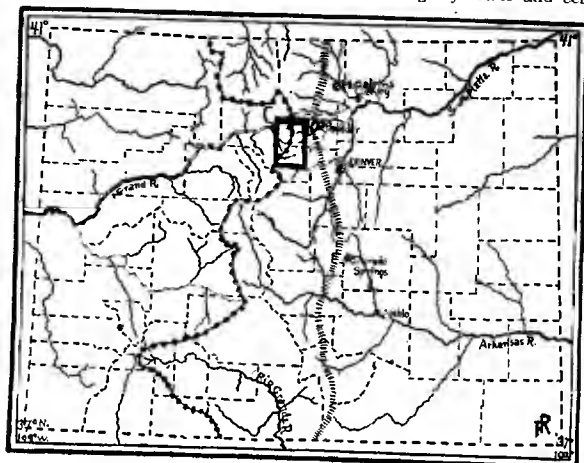
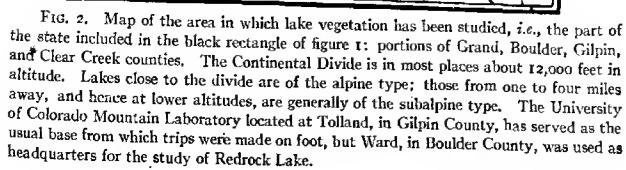


FIG. 1. Key map of Colorado. The Continental Divide is shown by the heavy dotted line, the front range of foothills by the short horizontal lines. The black rectangle outlines the area in which lake vegetation has been studied. Immediately north of this area is situated the Rocky Mountain National Park (not indicated on the map), of about the same size and shape as the part here marked out.

peaks much higher (Mt. Audubon, 13,225; Arapahoe Peak, 13,506; James Peak, 13,260; Parry Peak, 13,345). Long ridges extend out peninsula-like from the divide. Between the ridges are deep valleys all or most of which held glaciers at various points during comparatively recent times. On Arapahoe Peak there is still a permanent glacier of considerable size. A mile to the north is a smaller one (Henderson Glacier) and two others, also small, the Fair Glacier and the Isabelle Glacier, are four miles farther. At the extreme northern limit of our area of study are two more, the St. Vrain Glaciers. The broader parts of valleys are from one half to three quarters of a mile in width, and each may hold a group of small lakes, sometimes six or more. Along the flanks of ridges and on the slopes of the divide there are numerous cirques each with a single small lake.

The higher lakes are chiefly of the rock-basin type while those of the subalpine zone are usually morainal. No ox-bow lakes are known to the writer above 10,000 feet altitude, although they occur in parks of the montane zone (8,500 to 10,000 feet).



## CLIMATE AND SOIL

The climate of the area under consideration is cold, corresponding to that of Labrador, the Hudson Bay country, and southern Greenland. The timber limit is about 11,500 feet; lakes above this datum I shall speak of as alpine, those between this altitude and that of 10,000 feet as subalpine.

At Corona, on Rollins Pass, at an altitude of 11,660 feet, a government weather-bureau station was maintained (3) for a number of years. Here the mean annual temperature was found to be 26 degrees F. and the precipitation 43.7 inches. These figures may be taken as representative of the alpine region in this part of Colorado, although the tops of the higher peaks are, no doubt, colder and wetter. Throughout the alpine district there is frost every month of the year and in many places nearly every day.

For the subalpine zone only very incomplete records are available, but it is likely that at 10,000 feet the mean annual temperature is about 36 degrees F. and the precipitation 30 inches. The period without frost does not exceed three or four weeks even in favorable seasons. Precipitation is always ample during the growing period. There are frequent light showers during July and August (8). In table 1 a comparison is made with various

TABLE 1. *Temperature and precipitation in the subalpine and alpine zones of Colorado compared with data from various points in the northern United States. Temperature in degrees Fahrenheit; precipitation in inches*

Station	Mean Annual Temperature	Mean July Temperature	Mean Annual Precipitation
Subalpine zone (10,500 ft.)	34.0 <sup>1</sup>	54.0 <sup>1</sup>	32.0 <sup>1</sup>
Alpine zone (Corona, 11,660 ft.)	26.0	47.0	44.0
Denver, Colo. (5,275 ft.)	49.8	71.8	14.0
St. Paul, Minn.	45.0	74.0	28.6
Duluth, Minn.	39.0	66.0	29.9
Chicago, Ill.	48.0	72.0	33.4
New York, N. Y.	52.0	74.0	44.8

points in the northern United States so that a clearer idea of the climate of our area of study may be gained.

The temperature of the soil, as would be expected, is low. Numerous observations have been made at subalpine lakes. Readings at 3 dm. depth are shown in table 2. In analogous associations at ordinary altitudes in the northern United States the temperatures are 10 to 18 degrees higher.

TABLE 2. *Soil temperatures of subalpine lake shores for July; average of numerous observations at 3 dm. depth, in degrees F.*

Subalpine sedge moor, near water	50 <sup>1</sup>
Subalpine meadow, on higher ground	52
Subalpine spruce forest (dense)	48
Subalpine forest openings (dry places)	60

<sup>1</sup> Data very meager; estimated by comparison with stations in the alpine and montane zones.

Soils throughout the area studied are derived primarily from granitic rocks. On many ridges the material is a compact disintegrated granite, and the shores of subalpine lakes are often of this material interspersed with large and small boulders. At inlets and outlets, and wherever an accumulation of wash from adjacent slopes occurs, the soil is a black loam.



FIG. 3. Part of Corona Lake (altitude 11,165 feet), a high subalpine lake without any complete circum-areas of vegetation. Numerous large rocks are to be noted along the shore which show that there has been very little infilling. In the lower right-hand corner of the picture an Engelmann spruce is seen; behind this is a clump of willows; farther around is sedge moor; then more willows.

Here sedge moor and willow moor (willow scrub) develop. Typical subalpine meadow is found on lighter, better-drained soil, a sandy loam which occurs often as a circum-area of lakes between the moor and the forest.

Soil moistures have not been so fully determined as soil temperatures. Figures for July, 1918, at Redrock Lake (altitude 10,100 feet) are, however, available (9). They indicate an abundance of moisture. Averages are shown in table 3.

TABLE 3. *Soil moisture percent at 3 dm. depth during July, 1908, at Redrock Lake in the subalpine zone*

Subalpine sedge moor, near water.....	65
Subalpine meadow, on higher ground.....	21
Subalpine spruce forest (dense).....	29
Subalpine forest openings (dry places).....	7

No studies of wilting coefficients have been made, but so far as the writer's observations go there is little wilting of vegetation even in the driest weather.

Probably the chief limiting factors for plant growth around subalpine lakes are low temperature, extreme shortness of season, and shallow soil.

At very high altitudes there can be no doubt that the drying effect of winter winds and the heavy snows are important in preventing forest development.

#### PLANT COMMUNITIES OF LAKE SHORES

##### Types of Zonation

Great similarity exists in the shore vegetation of the various lakes studied. It is convenient, as already suggested, to separate subalpine and alpine life-zones by an arbitrary datum of 11,500 feet altitude. There is, however, no sharp difference in vegetation immediately below and above this line, yet it is possible to distinguish a subalpine and an alpine type of lake.



FIG. 4. One of the larger Forest Lakes (altitude 10,800 feet), showing coniferous forest of Engelmann spruce coming down close to the lake edge. There has been very little infilling either through wash from the slopes or through accumulation of plant remains. At the left there is a narrow fringe of moss moor; on the shore opposite the observer a considerable amount of meadow moor has developed in the lower places close to shore. Photograph in early June by Dr. W. W. Robbins.

The former only will be considered in the present paper. So far as the writer is able to do so, he will use a terminology consonant with that proposed by Nichols (4). Plant nomenclature will be that of Rydberg (12).

Subalpine lakes are typically surrounded by Engelmann spruce forest in which subalpine fir (*Abies lasiocarpa*) and lodgepole pine (*Pinus murrayana*) may occur in small amount. Aspens (*Populus tremuloides*) are occasionally present, but they belong rather to the montane zone where they are very abundant. On the eastern wind-swept shores of lakes the forest is often made up largely of limber pines (*Pinus flexilis*), the trees scattered with intervening open spaces.

Lakes shut in by steep slopes often show no special shore vegetation. The Engelmann spruce forest extends down close to the lake edge, and here there may be a zone of rocks with almost no vegetation at all. This condition obtains particularly where the lake level fluctuates from year to year or in different months of the same season. Most lakes have some parts of the shore either barren or else covered with forest. Lakes which show no true shore vegetation furnish nothing for discussion in the present paper.



FIG. 5. One of the small Forest Lakes (altitude 10,800 feet); a shallow pond with much infilling and showing complete circum-areas of moss moor and sedge moor. A half submersed belt of *Carex aquatilis* runs about one third of the way around the lake. Nearly every one of the various lake-shore associations is represented in some part of the area surrounding this lake. Photograph by Dr. W. W. Robbins.

Many lakes have gently sloping banks for a part of their circumference. On these more moderate slopes a true shore vegetation develops, determined by differences from the forest in soil quality, soil moisture, and soil temperature. A common arrangement of the vegetation of these lakes is in three clearly-marked circum-areas (8). As a rule, some of the associations are absent at certain points. Willows may be present only near the lake inlet and outlet (see table 4).

TABLE 4. *Subalpine shore vegetation; condensed classification*

1. Moor; next to water:
  - a. Sedge moor; chiefly *Carex*, with *Caltha*, *Bistorta*, *Clematis*, and other marsh plants.
  - b. Willow moor (willow scrub); farther from the water but the soil very wet. The herbaceous vegetation is chiefly *Carex* with some shade-enduring grasses and dicotyledons.
2. Meadow; on drier ground, but the soil fairly deep and of moderately fine texture. Often this is a close association of many species of flowering herbs.
3. Forest; Engelmann spruces chiefly, if the soil is deep, but lodgepole pine and limber pine on steeper slopes and on ridges with scanty soil.



In favorable parts of subalpine lake shores a closer analysis of the vegetation can be made than is indicated in table 4. Thus the moor is seen to include as many as six communities arranged in successive belts, a heath association may be distinguished outside the moor, *i.e.*, in drier ground; two or more consociations may form distinct bands in the meadow association. All of these communities are associated with differences in edaphic conditions and are not merely floristic in nature. Such a vegetation complex is indicated in table 5.

TABLE 5. Subalpine shore vegetation, extended classification

1. Moor (moor-association type):
  - a. Half submersed association of *Carex aquatilis*.
  - b. Moss-moor consociation; sedge moor with large amount of moss.
  - c. Typical sedge-moor association.
  - d. Willow-moor association; sedge moor with shrubby willows.
  - e. Rush-moor society; sedge moor with rushes (*Juncus Drummondii* and *Juncus mertensianus*).
  - f. Meadow-moor consociation; sedge moor with a number of meadow plants and hence a smaller proportion of *Carex* than typical sedge moor.
2. Heath association:
  - a. Heath moor, a transition between heath and moor.
  - b. *Kalmia* heath consociation.
  - c. *Gaultheria* heath consociation.
3. Meadow association:
  - a. *Erigeron*-*Castilleja*-*Ligusticum* consociation.
  - b. *Pedicularis*-*Vaccinium* consociation.
4. Forest association.

It seldom occurs that all these communities can be distinguished for any great distance along the shore. Many lakes have a part of the shore in which the vegetation analysis may be carried as far as indicated in our table, other parts of the shore may show no more than is suggested in table 4, while still other parts have no distinct shore vegetation at all. An abnormal position of some of the communities is often brought about, due to local areas of seepage. Islands of meadow moor occur in many places surrounded by sedge moor, while similar islands of willow moor and moss moor are common (see map, fig. 6). Meadow moor, rush moor, and the entire heath association are absent from many lakes. The positions of heath and meadow are sometimes completely reversed, or there may be heath meadow in which there is a mingling of plants of the two associations. Lakes near to timber limit may have little vegetation except meadow moor, or sedge moor and meadow moor.

#### Descriptions of the Various Lake-shore Zones (Circum-areas)

The half submersed *Carex aquatilis* association is typically a pure stand of the species of *Carex* which gives its name. This is in contrast to conditions in montane lakes (5, 7, 11), where three or more species may make up the half submersed zone.

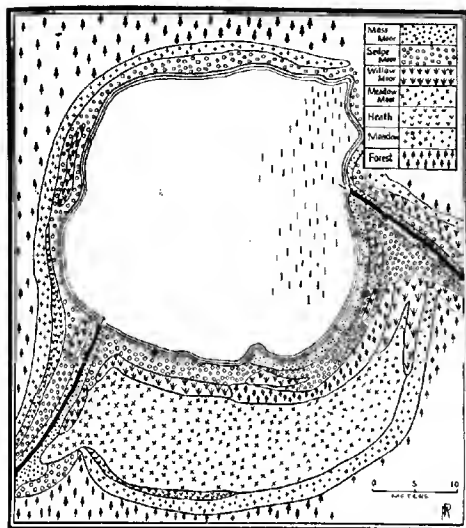


FIG. 6. Map of vegetation at Burgrass Lake, one of the Forest Lakes, a small sub-alpine pond at an altitude of 10,800 feet. Scattered plants of burgrass (*Sphagnum angustifolium*) at the east side near the outlet give the lake its name. Rush moor and heath moor, imperfectly developed in places, could not well be shown on the map.

Typical *sedge moor* has often been described (5, 7, 9). *Moss moor* and *true sedge moor* may be distinguished in the following quadrat records in which the percentage of ground covered by each kind of plant is indicated (tables 6 and 7). Naturally, any quadrat in a community would be different from every other quadrat as to minute details, but quadrat records afford the best means of distinguishing similar communities.

TABLE 6. Meter quadrat in subalpine moss moor at Burgrass Lake (one of the Forest Lakes) in Gilpin County, Colorado, August 17, 1918. The figures indicate percentages of ground covered

Bare ground	0
Mosses	40
<i>Carex aquatilis</i>	40
<i>Clementsia rhodantha</i>	5
<i>Ligusticum tenuifolium</i>	5
<i>Caltha rotundifolia</i>	5
<i>Salix chlorophylla</i>	2
<i>Viola palustris</i>	1
<i>Epilobium anagallidifolium</i>	1
<i>Dodecatheon radiculatum</i>	1

100

TABLE 7. Meter quadrat in true subalpine sedge moor at Burgrass Lake (one of the Forest Lakes) in Gilpin County, Colorado, August 17, 1918.  
The figures indicate percentages of ground covered

Bare ground	0
<i>Carex aquatilis</i>	60
Mosses	15
<i>Carex nigricans</i> and <i>C. illota</i>	5
<i>Caltha rotundifolia</i>	5
<i>Elephantella groenlandica</i>	5
<i>Ligusticum tenuifolium</i>	5
<i>Salix</i> spp.	4
<i>Viola palustris</i>	1
	<hr/> 100

Willow moor, or willow scrub, as previously suggested, is sedge moor with willows in it. The writer has usually thought of it as quite distinct from sedge moor, but on subalpine lake shores no sharp distinction can be drawn. It does not form, as a rule, a clearly marked circum-area, as is common in the montane region below 10,000 feet. Where present at subalpine lakes, willow moor is often a stage preceding the development of Engelmann spruce forest. This is especially true in late stages of infilling, when a whole lake may become willow moor and, later, spruce forest. Willows are most likely to develop on deep fine-grained soil. The species are *Salix chlorophylla*, *S. glaucops*, *S. lutea*, and *S. Barclayi*.

Rush moor is a society of sedge moor. On many shores it is very conspicuous as a narrow belt outside the ordinary sedge moor, most often when willows are absent. The usual species of rush are *Juncus Drummondii* and *J. mertensianus*, with, sometimes, *Juncoides spicatum*.

Meadow moor is sedge moor with some meadow plants. It may form a definite belt between moor and meadow, but more often it occurs as large or small patches developed on soil somewhat elevated above ordinary sedge moor and hence better drained. According to the writer's "soil moisture index" (6), ordinary xerophytes are given the number 4, mesophytes 6, marsh plants 8, and aquatics 10. Meadow moor would be assigned 7 as its index number. It is sometimes convenient to call it a "no. 7 meadow." There is less sedge and especially less moss than in ordinary sedge moor. More different species of plants occur than in ordinary sedge moor or in moss moor. The following are likely to be present in considerable amount: *Ligusticum tenuifolium*, *Erigeron salsuginosus*, *Bistorta bistortoides*, *Arnica fulgens* and *A. subplumosa*, *Deschampsia atropurpurea*, *Senecio blitoides*. Besides these, almost any of the true meadow plants may occur.

The heath association is here a mere suggestion of the heaths so prominent in the shore vegetation of many lakes in the northeastern United States and in Canada. The writer designates as "heath moor" the transition between moor and heath. This may be a distinct belt in which plants of

the two associations are mingled, or it may be entirely lacking. In the latter case the belt of heath may follow abruptly after sedge moor or rush moor or meadow moor. It may be, in turn, followed on higher ground by forest, or there may be an intervening strip of meadow. Heath may occur in scattered patches on elevated areas of the sedge moor, probably most often in shallow soil over large rocks. A quadrat record (table 8) taken in a part of the heath which is not differentiated into consociations gives an idea of the floristic composition.

TABLE 8. Meter quadrat in subalpine heath association at North Forest Lake (altitude 10,800 ft.) in Gilpin County, Colorado, August 17, 1918.  
The figures indicate percentages of ground covered

Bare ground and rocks	20
<i>Gaultheria humifusa</i>	25
<i>Vaccinium caespitosum</i>	18
<i>Kalmia microphylla</i>	15
<i>Erigeron salsuginosus</i>	7
<i>Carex festuella</i> et spp.	5
<i>Ligusticum tenuifolium</i>	4
<i>Juncus Drummondii</i>	2
<i>Agrostis humilis</i>	2
Lichens	2
	<hr/> 100

The *Kalmia* heath consociation is characterized by the low shrub *Kalmia microphylla*, about 2 dm. tall. This is often only scantily distributed, but sometimes it forms a clearly-marked though narrow belt of vegetation part way around the moor. The writer has, in no case, seen a complete circum-area of this plant.

The *Gaultheria* heath consociation seems to develop often on shallow soil. In it dense patches of *Gaultheria humifusa* occur. The plant is a depressed undershrub only a few centimeters high. Subordinate species are *Vaccinium caespitosum*, *Muhlenbergia filiformis*, *Erigeron salsuginosus*, *Hieracium gracile*. Almost any meadow plant may occasionally be present.

The meadow association is conspicuous because of the large and brightly-colored flowers of some of the abundant species. It would be possible to name six or more societies of local or infrequent occurrence distinguished by floristic differences, but in the present paper it will be best merely to characterize two consociations. These, as already indicated in table 5, may be called the *Erigeron-Castilleja-Ligusticum* consociation and the *Pedicularis-Vaccinium* consociation. They are rather constant in occurrence, the first named being next to the heath (or to sedge moor or rush moor or meadow moor in some cases), the second merging into the undergrowth of the forest. There may be considerable bare ground (10 to 40 percent). Many species characteristic of lake-shore meadows in the montane zone (10, 11) are absent, as *Fragaria glauca*, *Tium alpinum*, *Potentilla pulcherrima*, *Erigeron macranthus*.

The *Erigeron-Castilleja-Ligusticum* consociation of the meadow develops on fine-grained soil with abundant moisture, and well drained. The dominant species are *Erigeron salsuginosus*, *Castilleja confusa* and *C. laevis*, and *Ligusticum tenuifolium*. Among other plants are *Potentilla diversifolia*, *Amarilla strictiflora*, *Achillea lanulosa*, *Artemisia saxicola* and *A. scopulorum*, *Veronica Wormskjoldii*, *Phleum alpinum*, *Carex festivella*, *Bistorta bistortoides*, *Antennaria umbrinella*, and those mentioned in the following paragraph.

The *Pedicularis-Vaccinium* consociation is on drier ground than the community just described. *Pedicularis Parryi* and *P. Grayi* are important. *Vaccinium oreophilum*, a typical plant under Engelmann spruces, is usually present here at the edge of the forest. *Vaccinium scoparium* and *V. oreophilum* may also be present. All of these species of *Vaccinium* may at times be found sparingly in the Kalmia heath consociation. Many subordinate species of the *Pedicularis-Vaccinium* consociation are plants common in the forest or in forest openings, such as *Thlaspi Nuttallii*, *Chamaenerion spicatum*, *Koeleria gracilis*, *Micranthes rhomboidea*, *Aquilegia coerulea*. Then there are such meadow plants of the montane zone as *Dasystephana Parryi* and *Troximon glaucum*, and such plants of alpine meadow as *Trifolium dasyphyllum* and *T. nanum*. In addition there may be any of the species mentioned in the preceding paragraph.

TABLE 9. Meter quadrat in subalpine meadow at Burrass Lake (one of the Forest Lakes) in Gilpin County, Colorado, August 17, 1918. The figures indicate percentages of ground covered

Bare ground and rocks.....	10
<i>Ligusticum tenuifolium</i> .....	12
<i>Erigeron salsuginosus</i> .....	8
<i>Castilleja confusa</i> .....	7
<i>Castilleja laevis</i> .....	7
<i>Artemisia scopulorum</i> and <i>A. saxicola</i> .....	6
<i>Potentilla diversifolia</i> .....	5
<i>Arnica fulgens</i> .....	5
<i>Agrostis humilis</i> .....	5
Mosses.....	5
<i>Amarilla plebeja</i> .....	4
<i>Veronica Wormskjoldii</i> .....	4
<i>Vaccinium caespitosum</i> .....	4
<i>Hieracium gracile</i> .....	3
<i>Viola bellidifolia</i> .....	3
<i>Phleum alpinum</i> .....	3
<i>Sibbaldia procumbens</i> .....	3
<i>Trifolium dasyphyllum</i> .....	3
<i>Chamaenerion spicatum</i> .....	2
<i>Juncus Drummondii</i> .....	1

100

An idea of the composition of the meadow may be gained from a quadrat

cord at Burgrass Lake. Where this was taken the belt of meadow was narrow that the meter quadrat covered both consociations, and most of the common meadow plants are, therefore, represented. *Ligusticum*, it will be seen, occupied more space than any other species. *Castilleja* and *Ligeron* were, however, more conspicuous because of their brilliant flowers.

The forest association surrounding subalpine lakes does not differ from the forest elsewhere in the same locality. It is, therefore, not included in the present study.

#### LIST OF PLANT SPECIES

The following list includes only the more frequent plants of subalpine lake shores. Certain species may be locally abundant and yet not widely distributed. These have generally been excluded. Plants belonging primarily to the forest and only occasionally getting in among the true moor, peath, and meadow plants are also not admitted, nor have aquatics been listed. A number of species characteristic of stream banks and of narrow gulches, as *Cardamine cordifolia*, *Heracleum lanatum*, *Primula Parryi*, *Fertensia ciliata*, and *Senecio triangularis*, are occasional in the moor but are not included in the list. Mosses, lichens, and fungi are not considered.

Many plants are lacking which are characteristic of lake shores at lower altitudes in Colorado and at ordinary altitudes in the United States east of the Rocky Mountains. The following may be mentioned: *Equisetum*, *Cyperus*, *Alisma*, *Beckmannia*, *Panicularia*, *Cyperus*, *Scirpus*, *Iris*, *Populus*, *Lythrum*, *Persicaria*, *Rumex*, *Thalictrum*, *Rosa*, *Lathyrus*, *Vicia*, *Euphorbia*, *Penstemon*, *Mentha*, *Prunella*, *Galium*, *Sambucus*, *Aster*, *Bidens*, *Iva*, *Achillea*, *Solidago*, *Taraxacum*. Some few of the above named are found in the shore vegetation of subalpine lakes close down to the 10,000-foot-altitude line, as *Equisetum*, *Betula*, *Rosa*. They do not, however, belong to typical subalpine lakes.

Since the soil moisture requirement is the most useful single feature to be known about a plant, provided the general climatic features of the region are known, this has been indicated in the list. The plan followed is that employed by the writer (6) and by one of his students (10) whereby, as previously stated, the figure 4 is used as the "soil moisture index" for ordinary xerophytes, 6 for mesophytes, 8 for marsh plants, 10 for aquatics. Most plants of sedge moor have a soil moisture index of 8, most plants of meadow have a soil moisture index of 6. Species which grow under various conditions are given more than one number.

#### POACEAE

<i>Agrostis humilis</i> (6, 7)	<i>Muhlenbergia filiformis</i> (6, 7)
<i>Lopecurus occidentalis</i> (7, 8)	<i>Phippsia algida</i> (7)
<i>Deschampsia alpicola</i> (6, 7, 8)	<i>Phleum alpinum</i> (7)
<i>Deschampsia caespitosa</i> (7, 8)	<i>Poa alpina</i> (7, 8)
<i>Poa gracilis</i> (5, 6)	<i>Poa subpurpurea</i> (5)
	<i>Sporobolus brevifolius</i> (6)

## CYPERACEAE

- Carex albo-nigra* (7, 8)  
*Carex aquatilis* (8, 9)  
*Carex ebenea* (7)  
*Carex festivella* (6)  
*Carex nigricans* (6, 7, 8)  
*Carex pyrenaica* (6)  
*Carex rostrata* (8, 9)  
*Carex scopulorum* (8)

## JUNCACEAE

- Juncoides spicatum* (5, 6, 7)  
*Juncus Drummondii* (6, 7)  
*Juncus merlensianus* (7, 8)

## SALICACEAE

- Salix Barclayi* (8)  
*Salix chlorophylla* (8, 9)  
*Salix glaucops* (7, 8)  
*Salix lutea* (8)

## POLYGONACEAE

- Bistorta bistortoides* (7, 8)  
*Bistorta vivipara* (7, 8)

## ALSINACEAE

- Alsine longifolia* (7)

## RANUNCULACEAE

- Aquilegia coerulea* (6)  
*Caltha rotundifolia* (7, 8, 9)  
*Ranunculus alismaefolius* (8)  
*Ranunculus alpeophilus* (7, 8)  
*Trollius albidiflorus*

## BRASSICACEAE

- Draba Parryi* (5, 6)  
*Thlaspi Nuttallii* (5)

## CRASSULACEAE

- Clematis rhodantha* (8, 9)

## SAXIFRAGACEAE

- Micranthes arguta* (8, 9)  
*Micranthes rhomboidea* (6)

## ROSACEAE

- Dasiphora fruticosa* (5, 6, 7)  
*Potentilla diversifolia* (7)  
*Sibbaldia procumbens* (6)

## FABACEAE

- Trifolium dasyphyllum* (5, 6)  
*Trifolium nanum* (5, 6)  
*Trifolium Parryi* (5, 6)

## VIOLACEAE

- Viola bellidifolia* (6)  
*Viola palustris* (8)

## ONAGRACEAE

- Chamaenerion spicatum* (5, 6)  
*Epilobium alpinum* (8, 9)  
*Epilobium anagallidifolium* (8, 9)  
*Epilobium Hornmannii* (8)

## AMMIACEAE

- Angelica Grayi* (8)  
*Ligusticum tenuifolium* (7, 8)  
*Oxyopsis Fendleri* (7, 8)

## ERICACEAE

- Gaultheria humifusa* (6, 7)  
*Kalmia microphylla* (7)

## VACCINIACEAE

- Vaccinium caespitosum* (5, 6)  
*Vaccinium oreophilum* (5, 6)  
*Vaccinium scoparium* (5, 6)

## PRIMULACEAE

- Androsace subumbellata* (6)  
*Dodecatheon radiculatum* (8, 9)

## GENTIANACEAE

- marella monantha* (8)  
*marella plebeja* (7)  
*marella strictiflora* (7, 8)  
*hondrophylla Fremontii* (8)  
*vasystephana Parryi* (6)  
*vasystephana Romanzovii* (6, 7)  
*leurogyne fontana* (8)  
*wertia scopulina* (8, 9)

## SCROPHULARIACEAE

- astilleja confusa* (6)  
*astilleja laeta* (7)  
*elephantella groenlandica* (8)  
*edicularis Grayi* (6)  
*edicularis Parryi* (6, 7)  
*edicularis racemosa* (5, 6)  
*pentstemon stenosepalus* (6)  
*veronica Wormskjoldii* (8)

## CAMPANULACEAE

- Campanula petiolata* (5, 6, 7)

## CARDUACEAE

- Achillea lanulosa* (5, 6, 7)  
*Antennaria umbrinella* (6)  
*Arnica fulgens* (6)  
*Arnica Parryi* (6)  
*Arnica subplumosa* (6)  
*Artemisia saxicola* (6)  
*Artemisia scopulorum* (6)  
*Erigeron salsuginosus* (6, 7)  
*Oreochrysum Parryi* (5, 6)  
*Senecio blitoides* (6)  
*Senecio crassulus* (6, 7)

## CICHORIACEAE

- Agoseris glauca* (5, 6)  
*Hieracium gracile* (6)

## SEASONAL ASPECTS

The growing period of subalpine lake-shore vegetation may be separated into three seasons: vernal (May 15 to July 1), estival (July 1 to August 5), autumnal (August 15 to October 1).

During the vernal period there are few lake-shore plants in bloom. About June 1 or a little later the willow catkins appear. The brown of winter continues, especially at higher altitudes, until the middle of June. There are still large snowdrifts in the forest and often some in the open. It must not be thought that there is no fresh vegetation in the subalpine zone at this time. A number of xerophytic plants of ridges and dry slopes and forest openings are in bloom, but these do not belong to our present study. Of lake-shore plants, in addition to the willows already mentioned, the spring bloomers are *Caltha*, *Trollius*, *Viola bellidifolia*, *V. palustris*, *Thlaspi Nuttallii*, *Draba Parryi*. *Caltha* and *Trollius*, on account of their abundance and their showy flowers, are the conspicuous plants of the spring season.

During the estival period the great majority of lake-shore plants come into blossom. Many of the sedges are rather early; grasses are somewhat later. The spring flowers continue for a time but are soon overshadowed by the abundance and brilliancy of the summer bloomers. In the moor there are *Clementsia*, *Dodecatheon*, *Elephantella*, *Dasiphora*, *Swertia*, and *Senecio*. Among meadow and heath plants may be mentioned the *Castillejas*, the *Arnica*s, *Potentilla diversifolia*, *Kalmia*, *Campanula*, *Erigeron salsuginosus*, *Hieracium gracile*.



During the autumnal period the summer-blooming plants are in fruit and a few of them continue to bloom for a time, especially *Erigeron saluginosus*, *Campanula petiolata*, the Castillejas, and the Arnicas. True autumn-blooming plants are the gentians Amarella, Chondrophylla, and Dasystephana, also *Antennaria umbrinella* and *Ligusticum tenuifolium*. The snows which usually come about the first week in September melt away in a few days, and some of the plants continue in bloom up to about September 20, or occasionally even to October 1.

#### SUCCESSIONS

The character of succession on the lake shores here described is so obvious that an extended discussion will not be necessary. It needs to be kept in mind that the topography of the area is "new." There is comparatively little soil anywhere except in depressions where it has been washed down from adjacent slopes. Lake bottoms are typically of boulders. Climate is of the cold, moderately wet type which favors the development of coniferous forest. Engelmann spruce forest is the ultimate climatic association and appears wherever edaphic conditions are at all favorable.

A few subalpine lakes, especially those at lower altitudes close to 10,000 feet, have a moderate amount of aquatic vegetation, chiefly *Potamogeton* spp. and *Sparganium angustifolium*. These plants contribute slightly to the filling up of lakes. At higher altitudes they are generally absent and the only lake vegetation of consequence is *Carex*, which may form in places a strip of half submersed plants extending into the water from the moor. The *Carex* here is commonly *C. aquatilis*, which is also the chief sedge of the moor that surrounds subalpine lakes.

Often the vegetation advances very slowly into the lake because of the heaving action of ice. This thrusts itself into the bank and destroys vegetation. It may give rise to an elevated "rim" as shown by Robbins (11), projecting out a few decimeters over the edge of the water.

Infilling of subalpine lakes is, it will be recognized, largely a physiographic process depending upon stream sediment chiefly near the inlet, or upon storm wash from surrounding slopes. When, however, a lake has once been filled so that the water is quite shallow, then a rapid invasion by *Carex* may occur.

The normal positions of the various plant communities show their successional relations. Half submersed *Carex* association is followed by moor, and this by heath and meadow and forest. When level ground is raised by accumulation of plant remains or by alluvial wash then a later successional stage of vegetation develops. The same change occurs when the lake level is lowered. One or more stages may be skipped.

Moss moor is usually the wettest part of the moor. As vegetation advances this becomes drier and changes to ordinary sedge moor or to meadow moor. The willow moor stage may follow sedge moor if there is a

and depth of fine-grained soil. While in montane situations willows tend to form a complete circum-area of lakes, they are relatively unimportant in the lake-shore vegetation of the subalpine region. Where they do occur they may initiate a shortened successional series, for they permit the establishment of Engelmann spruce without the intermediate heath and meadow stages.

"Dry forest" of limber pine and lodgepole pine does not follow meadow but belongs to the xerarch series, developing on ridges and on rough, stony ground. In time, except where winds are so severe as to blow away the humus, Engelmann spruce forest, as the ultimate climatic association, will replace the dry forest.

#### SUMMARY

The subalpine zone, 10,000 to 11,500 feet in altitude, in north-central Colorado has a large number of small lakes, some of morainal, some of rock-basin type. These have a characteristic shore vegetation often developing definite circum-areas. The climatic climax association is Engelmann spruce forest, and the stages of succession in the filling up of a lake lead eventually to this forest. Very few aquatic plants occur, but there is sometimes a circum-area (usually incomplete) of half submersed sedges. Following this there may be in the more complete cases: (1) a well developed moor composed largely of *Carex*, sometimes separable into moss moor, sedge moor, willow moor, rush moor, and meadow moor; (2) a heath association of *Kalmia microphylla* and *Gaultheria humifusa*; (3) meadow association in which the principal plants are species of *Erigeron*, *Castilleja*, *Ligusticum*, *Pedicularis*, and *Vaccinium*; (4) forest association, dominated by Engelmann spruce. The various associations and their numerous subdivisions are described by the author together with their successional relations and seasonal aspects. A sketch is given of topography, climate, and soil, and a list is made of the characteristic lake-shore plants, with soil-moisture index of each. The paper is based upon the study of a large number of subalpine lakes in four different counties of north-central Colorado.

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## SOME OBSERVATIONS ON THE SPORE DISCHARGE OF PLEURAGE CURVICOLLA (WINT.) KUNTZE.

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### INTRODUCTION

While making some studies on the comparison of a strain of *Pleuraea curvicolla* (Wint.) Kuntze with those strains previously investigated, the results of which studies are being published in a separate paper,<sup>1</sup> it was noticed that this organism has the power to discharge its spores with remarkable force.

So far as known, parasitic Ascomycetes are able to project their spores only to a distance of a few millimeters, while it has been shown that certain saprophytic forms such as members of the Sordariaceae, Ascobolaceae, and others are able to shoot their spores a considerably greater distance. Griffiths<sup>2</sup> studied the method of spore discharge in *P. curvicolla* as well as in related species, and found that the maximum height of projection in the former is 9 centimeters. A good discussion of the details of spore ejection by these fungi is given by Griffiths and need not be repeated here. Woronin<sup>3</sup> found that *Sordaria fimiseda* could shoot its spores to a height of 15 centimeters. This is the maximum distance found recorded for a member of the Sordariaceae.

Buller<sup>4</sup> states that *Ascobolus immersus* is possibly not exceeded by any other Ascomycete in the violence of its spore discharge. It was found to project its spores to a height of 35 centimeters.

### EXPERIMENTS

In the experiments recorded below, the fungus was grown on moistened corn meal in a two-liter Erlenmeyer flask. The cultures were allowed to grow at room temperature, about 24–28° C., and exposed to the diffused light of the laboratory until the perithecia began to discharge their spores as indicated by the presence of the spore masses upon the sides of the flask. It might be mentioned in passing that few and often no perithecia were

<sup>1</sup> Weimer, J. L. Variations in *Pleuraea curvicolla* (Wint.) Kuntze. Amer. Journ. Bot. 6: 406–409. 1919.

<sup>2</sup> Griffiths, David. The North American Sordariaceae. Mem. Torrey Club 11: 1–134. 1901.

<sup>3</sup> Woronin, M. *Sphaeria Lemnaceae*, *Sordaria fimiseda*, *Sordaria coprophila* und *Arturobotrys oligospora*. Abhand. Senkenb. Naturforsch. Gesell. (Frankfurt) 7: 325–360. pl. 1–6. 1869–1870.

<sup>4</sup> Buller, A. H. R. Researches on fungi. pp. 287. 1909.

found in cultures grown in an incubator in the dark or wrapped in black paper, while in duplicate cultures grown in the laboratory in the light, perithecia were formed in abundance. When the culture was ready for use the cotton stopper was removed and a large test tube, 37 mm. in inside diameter and 243 mm. in height, was inverted over the mouth of the flask. The flask and all but the bottom of the tube were covered with black paper, to keep out the light. The apparatus was placed upon a desk before a north window, where it was exposed to rather strong diffused light. The perithecia are positively heliotropic, and hence the spores were discharged towards the source of light, namely, the bottom of the test tube. Spore masses were found all along the sides of the test tube to a height of 45 cm. above the fruiting surface of the culture. None were found on the bottom of the tube 5 cm. higher. This is three times as high as previously recorded for a member of this family, and 10 cm. higher than given by Buller for *Ascobolus immersus*. So far as the writer has knowledge, this strain of *Pleuroge curvicolle* can project its spores to a greater height than any other Ascomycete yet studied and three times as high as any other Pyrenomycete investigated.

Considerable attention has been paid by various workers to the study of the influence of light upon the direction in which spores are discharged. Allen and Jolivette<sup>5</sup> have reported in detail, observations made on the accuracy with which *Pilobolus* could project its sporanges toward a source of light. While endeavoring to determine the height to which *Pleuroge curvicolle* could shoot its spores, the writer incidentally made a few observations on its power to aim towards the light. Spore masses have often been seen on the side of the petri dish in which this organism was growing next to the source of light. In most cases perithecia were present only at or near the center of the culture, and these had oriented their beaks in such a way as to cause the spores to be discharged in a direction parallel to the surface of the substratum on which they were growing.

In one experiment a two-liter flask containing the fungus growing on corn meal was wrapped in two thicknesses of black paper, and a hole about  $2\frac{1}{2}$  cm. in diameter was cut in the paper on the side of the flask nearest the source of the strongest light. After 48 hours a considerable deposit of spore masses was present on the side of the flask immediately beneath the opening in the paper. The flask was then turned about so that the hole in the paper was directly opposite where it had been. This would mean, of course, that many of the beaks of the perithecia were pointing directly away from the opening. After a period of 48 hours a considerable deposit of spores was present beneath the opening in the paper. The paper was moved in this manner time after time with the result that each time the perithecia changed their aim and discharged their spores towards the source of light.

<sup>5</sup> Allen, Ruth F., and Jolivette, Hally D. M. A study of the light relations of *Pilobolus*. Trans. Wisc. Acad. Sci., Arts, and Lett. 17: 533-598. 1914.

In another experiment the opening in the paper was  $1\frac{1}{2}$  cm. in diameter and about 5 cm. from the bottom of the flask. The spore masses were found mostly within this circle, but a few were scattered in all directions about the opening; however, they were all included within a circle  $4\frac{1}{2}$  cm. in diameter concentric with the opening in the paper. The flask was then turned about so that the opposite side was exposed to the light through the opening in the paper. After 45 hours the paper was removed, and the same conditions as described above with regard to the arrangement of the spores about the opening were found to have been duplicated. In one experiment a grayish-black, instead of a jet-black, paper was used to exclude the light. In this case the spores, instead of being discharged towards the opening in the paper, were discharged against the side of the flask almost opposite the opening. This paper behind the glass acted as a mirror and it would seem that the reflected light exerted a stronger heliotropic influence than did the direct light. In this case the spore print covered an area on the side of the flask opposite and slightly below the source of light about six times larger than the area of the opening in the paper. The flask was then turned about, exposing the opposite side to the light, and the spores were again shot away from the direct light. However, when the paper was removed entirely the spores were discharged towards the window.

Buller shows that the great distance to which *Ascobolus immersus* spores are projected in comparison with that traveled by the spores of the Hymenomycetes is due to the large size of the spore mass. The size of this spore mass, he states, is due (1) to the unusually large size of the spores; (2) to the thick gelatinous envelope round each spore; (3) to the clinging together of the spores; and (4) to the large mass of the discharged ascus sap. In the case of *Pleuraea curvicolla* there are approximately 500 spores all clinging together and discharged as one body together with a quantity of ascus sap or other gelatinous substance, making a large projectile. A number of spore prints made upon a microscopic slide placed over the mouth of the two-liter culture flask 26 cm. above the fruiting surface were measured. These were circular in outline and ranged from 168 to 266  $\mu$  in diameter. Surrounding each spore mass was a sort of halo about 25 to 30  $\mu$  wide due to the gelatinous substance in which the spores were imbedded and which was discharged along with them. No doubt the comparatively great mass of material discharged in this instance, as in the case of *Ascobolus immersus*, is a big factor in determining the distance to which the spore masses are shot.

*Pleuraea curvicolla* probably can project its spores to a greater height than any other Ascomycete yet studied. Its spores are usually discharged in masses towards the source of light, but reflected light seems to exert a stronger heliotropic stimulus than does direct light.

CORRELATION BETWEEN SIZE OF THE FRUIT AND THE  
RESISTANCE OF THE TOMATO SKIN TO PUNCTURE  
AND ITS RELATION TO INFECTION WITH  
MACROSPORIUM TOMATO COOKE

J. ROSENBAUM AND CHARLES E. SANDO

That artificial infection with *Macrosporium tomato* Cooke<sup>1</sup> from tomato on uninjured tomato fruit can be obtained, provided fruit of a certain maturity as measured by size is used, has been established by one of the writers.<sup>2</sup> The question naturally arises as to the cause of this apparent immunity or resistance in the fruit after it reaches a certain maturity.

Previous investigations along these lines have been recently reviewed and summarized by Hawkins and Harvey<sup>3</sup> as follows: "It is apparent that there is good evidence that some parasitic plants make their way into their host plants by breaking through the tissues mechanically. There is no doubt that some fungi secrete enzymes which break down the cell walls of certain plants and are thus able to make their way through the tissues of their hosts."

The same workers from their infection studies with *Pythium* on potato conclude: "There is considerable evidence that the main factor in this penetration is the growth pressure of the fungus filament, and the resistance of the white McCormick potatoes to this disease is due to cell walls that are more resistant to mechanical puncture than are the cell walls of extremely susceptible varieties."

The results obtained by Blackman and Welsford<sup>4</sup> in their studies with *Botrytis cinerea* on *Vicia Faba* are of special interest. They state that "the piercing of the cuticle is due solely to the mechanical pressure exerted by the germ tube as a whole or by the special outgrowth from it."

In the work reported in this paper the evidence obtained shows that:

1. While a chemical difference is found in the analysis of young and old fruits, this is not the limiting factor in infection with *Macrosporium*. The

<sup>1</sup> The fungus causing typical "nail-head" spots on tomatoes has been shown in a paper which is being prepared for publication to be different from *Macrosporium solani* E. and M. For reasons given there this *Macrosporium* should be referred to as *Macrosporium tomato* Cooke.

<sup>2</sup> Rosenbaum, J. *Macrosporium solani* on tomato fruit (Abstr.). *Phytopathology* 9: 51. 1919.

<sup>3</sup> Hawkins, L. A. and Harvey, R. B. Physiological study of the parasitism of *Pythium debaryanum* Hesse on the potato tuber. *Journ. Agr. Res.* 18: 275-297. 1919.

<sup>4</sup> Blackman, V. H., and Welsford, E. J. Studies in the physiology of parasitism. II. Infection by *Botrytis cinerea*. *Annals of Botany* 30: 389-398. 1916.

fungus grows just as readily on the pulp and extracts of old fruits as on those obtained from young tomatoes. Moreover, positive infection has been obtained on fruits of all degrees of maturity when the skin is injured or removed previous to infection.

2. Surface sections of old and young fruits failed to reveal the presence of stomata or other natural openings in the skin.

3. As the tomato fruit develops, the surface of the fruit changes from a dull to a shiny appearance. The chemical nature of this change has not been determined, but sections cut from old and young tomatoes show that the cuticle increases in thickness with the age of the fruit. The development of the cuticular layer may be at least a partial explanation of the resistance of mature fruit to infection. Dewdrops are more readily retained on the dull surfaces than on the shiny and mature surfaces.

4. The maturity of the fruit as measured by size is correlated with a definite resistance of the tomato skin to puncture. The latter may also be one of the limiting factors in securing infection with *Macrosporium* on tomato fruit.

The methods employed in arriving at these conclusions and the detailed data obtained in this connection were as follows:

The tomatoes were grown in a commercial way in the fields of southern Florida. The work was limited to one variety, the "Livingston Globe." At first fruits of various sizes were selected at random. In order to get a more accurate knowledge of the fruit used, a large number of blossoms were tagged and pickings for stabbing and inoculations were made from these tagged blossoms at the end of each week. In this way it was possible to tell exactly the age of the fruit used, from blossoming time until the fruit began to show color in the field.

The *Macrosporium* cultures used in this work were isolated from tomato fruit. They were kept in pure culture and spores obtained according to the method described by Kunkel.<sup>5</sup> In a few cases spores were taken from fruit naturally infected in the field.

The resistance of the skin of the fruit to puncture was determined by the use of the Joly balance as modified by Hawkins and Harvey (*l. c.*). The construction of the Joly balance need not be given, but certain modifications of the balance and the methods followed in calculating the results obtained in the use of this apparatus in the present work will not be out of place here.

In using this apparatus for determining the resistance of the tomato skin, a fine glass needle 78 microns in diameter fixed to a glass rod with wax was suspended from the bottom of the pan. This needle was used throughout the experiments except in one instance as indicated. The needle and rod were well within the capacity of the spring of the balance. In operation the

<sup>5</sup> Kunkel, L. O. A method of obtaining abundant sporulation in cultures of *Macrosporium solani* E. & M. Brooklyn Botanic Garden Mem. 1: 306-312. 1918.



tomato was placed on the stand of the instrument; the needle was lowered until it just touched the surface of the tomato and watched closely until a quick drop showed that it had penetrated the skin of the fruit. The reading on the scale of the instrument was then taken. From this reading the pressure required to puncture the tomato skin could be calculated.

To illustrate the method of calculation, let us suppose the scale reading at which penetration took place on a fruit to be 31.00. The upward pull of the stretched spring representing this number as the reading on the scale was determined by counterbalancing with weights placed on the pan. This pull equalled 7.42 grams. The entire weight of the glass rod, with the needle, was 12.04 grams. The pressure necessary to puncture the skin was, therefore, the difference between the downward force and the upward force, or the difference between 12.04 and 7.42, or 4.62 grams. The pressure necessary, therefore, to penetrate the skin of a particular fruit with a needle which was 78 microns in diameter amounts to 4.62 grams.

Where tomatoes of different sizes were used, a total of five fruits of each size were punctured. Since the hardness over the entire fruit varies somewhat, it was thought advisable to make part of the punctures around the style end and an equal number around the stem end. Generally ten punctures were made on each fruit. The average of these readings gives fairly accurately the pressure necessary to puncture a particular fruit. As a general rule, it was found that the stem end was slightly harder than the style end. The fruits were picked and brought into the laboratory where they were divided into two lots, each lot containing fruits of the same maturity. One lot was used for determining the resistance of the skin to puncture while the other lot was washed, placed in disinfected moist chambers, and inoculated by spraying with a suspension of *Macrosporium* spores. In addition to the inoculations made on fruits brought into the laboratory, additional inoculations were made in the field on fruits growing on the vines. In this case the fruit was sprayed with a suspension of spores and covered with a glazed paper bag for a few days. No difference in the amount of infection was obtained whether the fruit was inoculated in the

TABLE 1. *Showing the Relation Between Resistance of the Skin to Puncture and Macrosporium Infection on Different Sized Tomatoes\**

Size	Color	Circumference in Inches	Average Weight in Grams	Pressure in Grams Necessary to Puncture Fruit (Average of 50 Stabs)	Percentage of Positive Infection with <i>Macrosporium</i>
	Red	.....	.....	5.19	0
A....	Green	10 3/4-11 1/4	254.87	5.87	0
B....	Green	7 3/4- 8 1/4	115.12	5.70	0
C....	Green	6 1/2- 6 3/4	66.36	4.08	37 1/2
D....	Green	5- 5 1/2	34.22	3.52	85 5/7
E....	Green	4- 4 1/2	18.17	3.26	72 8/11
F....	Green	3- 3 1/2	7.39	2.66	100

\* Temperature of tomatoes when punctured, 23° C.

Needle used, 78 microns in diameter.

laboratory or in the field. While a large number of punctures and inoculations were made throughout the season, the results are so uniform that it will suffice to present in tabular form a few representative series.

In Table 1 are shown the results of picking at random, at the same time, fruits of various sizes. These were divided into two equal lots, one of which was used for puncturing to determine the resistance of the skin, while the other was used for inoculations.

Examination of this table shows that the resistance of the skin to puncture increases with the size of the fruit, and likewise that the amount of infection varies from 100% in the case of the smallest fruit to 37½% in the case of fruit approximately 5-6 inches in circumference, with no infection above that size. From this series, the point at which the hardness of the skin begins to show any appreciable effect on infection is approximately that at which 4.08 grams of pressure is necessary to puncture the skin.

Table 2 shows the results of puncturing and inoculating fruits of known age for seven consecutive weeks. The fruits used in these series were all tagged when in blossom. The data show, as in the preceding table, that the older the tomato the more resistant is the skin, and that the amount of infection decreases when the resistance of the skin to puncture is approximately such that 5.08 grams of pressure is necessary to puncture the fruit with a 78-micron needle.

TABLE 2. *Showing the Relation Between Resistance of the Skin to Puncture and Macrosporium Infection on Tomatoes of Different Age*

Age in Days	Color	Weight in Grams, Average of 10 Fruits	Equatorial Diameter in Centimeters, Average of 10 Fruits	Temperature at which Scabbing was Done	Pressure in Grams Necessary to Puncture Fruit (Average of 100 Stabs)	Percentage of Positive Infection with <i>Macrosporium tomato</i>
7...	Green	0.24	0.70	..	0.97	100
14...	Green	6.74	2.30	21	2.99	100
21...	Green	64.66	5.18	25	4.21	85
28...	Green	82.37	5.49	22	4.90	49
35...	Green	95.10	5.46	21	5.08	23 1/3
41...	Green	147.91	6.55	21	5.96	0
48...	Green	91.86	6.92	23	6.74	0
55...	Turning	....	....	25	5.56	0
55...	Red	162.82	6.31	25	5.10	0

\* Size of needle used for 7-day-old fruit, 46 microns in diameter. At all other ages punctured a 78-micron needle was used.

Tagging of blossoms to obtain fruits of a known maturity has shown that in the majority of cases age of fruit is a better indication of maturity than is size. As would naturally be expected, not all the tomatoes in a given lot of fruits attain the same size in a given length of time. Such fruits, however, have a resistance of the skin in proportion to their age rather than to their size, and react accordingly when inoculated. For this reason then, it seems, resistance of the skin to puncture is a better index of maturity than is the size of the fruit. The former is also preferable in

predicting whether a certain tomato can or cannot be infected with *Macrosporium*.

#### SUMMARY.

In the development of a tomato fruit, the cuticular layer increases in thickness with the age of the fruit. Measurements to determine the resistance of the skin of tomatoes have shown that there is a definite and direct correlation between age and the resistance of the skin to puncture.

Infection experiments with *Macrosporium tomato* on tomato fruit have shown that the amount of infection which it is possible to obtain decreases with the age of the fruit.

While the results do not prove absolutely that the inhibition of infection is a purely mechanical one, the resistance of the tomato skin to puncture may explain, at least partially, the ease with which infection without previous injury is obtained on the young fruit but not on the older fruit.

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## THE LENGTH OF THE LIFE CYCLE OF A CLIMBING BAMBOO. A STRIKING CASE OF SEXUAL PERIODICITY IN *CHUSQUEA ABIETIFOLIA* GRISEB.\*

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Certain plants are known to live vegetatively for many years, then flower and die. The most frequently cited example of this phenomenon is that of the century plant, *Agave americana*, which lives for a period of years without flowering, then sends up a tall, prominent inflorescence, and finally, after the maturing of the seeds, dies. This sexual periodicity is also characteristic of certain bamboos which blossom only after a cycle of years and then all simultaneously throughout an extensive region. The bamboos in the South Brazilian provinces of Santa Catharina and Rio Grande do Sul are said to blossom at intervals of about thirteen years, and *Bambusa arundinacea* on the west coast of Cismagetic India blossoms at intervals of about thirty-two years (1). The complete and simultaneous dying off of the bamboos may in some communities prove disastrous by the wiping out of the chief available source of building material through the transformation of luxuriant bamboo forests into barren areas; or, it may prove of great economic value as a source of grain, especially when it comes, as it is said to (2), in times of drought and consequent famine.

The length of the interval of years varies greatly in different bamboos. Bean (3) reports that "*Bambusa tessellata* has been in cultivation for probably over sixty years, yet I have seen no record of its having flowered anywhere." In striking contrast with this is the case of *Arundinaria falcata* var. *glomerata* which flowers almost every year on a certain number of culms. The latter is a case of partial or sporadic flowering as contrasted with the complete and simultaneous flowering which is the rule among bamboos. Intermediate types also exist. Bean (3) mentions the case of *Arundinaria Simoni* which flowered on odd culms in the bamboo garden at Kew for several years. He says, "excepting that the flowering culms died, the plants were in no way affected. . . . They continued to flower in this way every year up to 1903, by which time we had almost come to regard *A. Simoni* as a perennial. In that year, however, the plants flowered on every culm, and, after producing an abundance of seed, died. After that

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